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THE EFFECT OF MICROORGANISMS ON PROCESSES OF WEATHERING PRIMARY MINERALS

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Observations of nature show that the surfaces of bare rocks, particularly those populated by lichens, support a varied microflora. /79*

This is indicated by investigations by V. O. Kalinenko (1932) and V. P. Tauson (1948) in the Pamirs, detailed studies by D. M. Novogradskiy (1949) of specimens of weathered granites from the Central Tien-Shan, and microbiological research by N. A. Krasil'nikov (1949) on various rocks from Armenia, and a series of other studies.

It is presently known that rock surfaces, even in the nival zone on mountains, is populated by a variegated flora of green and blue-green algae, while the weathered crusts and rock cracks are the habitat of numerous bacteria, fungi, and actinomycetes in addition to the algae.

A number of investigators note not only the presence of microorganisms on the rock surface, but also their erosive action on rocks, which is particularly great on limestones.

A number of experimental papers have studied the possibility of micro-organism breakdown of individual minerals.

Bassalik's studies in 1912 and 1913 demonstrated that certain bacteria are capable of decomposing orthoclases with the liberation of potassium and silica. Particularly powerful erosion of orthoclase (up to 3.5% of the original weight) was ascertained in cultures of Bacillus extorquens, which possess great respiratory energy.

V. I. Vernadskiy (1921) experimentally demonstrated that diatom algae of the genus Nitzschia can decompose kaolin. A. P. Vinogradov and Ye. A. Boychenko (1942) corroborated the capacity of algae to break down nacrite in a pure culture and in combination with azotobacter.

In the latter case [algae + azotobacter] the nacrite breakdown is considerably more intense.

Experimental research by B. B. Polynov and P. F. Martynov (1931) established the decomposition of silicates and aluminosilicates in connection with processes of desulfurization and subsequent oxidation.

While studying the genesis of podzolic soils, N. P. Remezov, L. Ye. Novorossova, and N. N. Sushkina (1947) discovered that they contained bacteria capable of decomposing aluminosilicates and silicates (microcline, kaolin, and, in particular, olivine).

* Note: Numbers in the margin indicate pagination in original foreign text.

In 1948 R. N. Pilovskaya found bacteria in phosphorites capable of converting calcium triphosphates into soluble forms.

By our own research (1950) it has been established that the surface of weathered rocks in the nival region of the Central Tien-Shan at absolute altitudes of 4000 meters and above is abundantly populated with microorganisms.

It seemed to us to be of interest to clarify the role played by this abundant and varied microflora in the processes of weathering primary minerals and the synthesis of secondary new formations. /80

Below we give the results of our experimental work conducted in the Laboratory of Soil Genesis of the Institute of Pedology of the Academy of Sciences, Kazakh SSR.

We performed the microbiological portion of the work in constant consultation with D. M. Novogrudskiy.

The chemical analyses, which demand great care in execution, were performed by V. S. Sukhenko. We express our sincere gratitude to these persons for helping in the work.

Problems, Materials, and Methods of Research

In setting up the experimental investigations of the effect of microorganisms on minerals, we endeavored to elucidate the following matters:

(1) Do microorganisms which are capable of actively affecting minerals and of extracting elements necessary to life from them, inhabit the surface of rocks in the nival region?

(2) If there are microorganisms of that sort, what groups of them exert the greatest erosive effect?

(3) Do prototrophic and oligonitrophyll microorganisms take part in this process?

(4) What are the minerals which are subject to the greatest degree of weathering from microorganism action?

For experimentation we chose minerals containing the various mineral elements necessary to the life of microorganisms. Orthoclase, muscovite, and biotite were utilized as the source of potassium; biotite and serpentine, as the source of magnesium. Of the minerals containing phosphorus we chose apatite, while the sulfur-containing minerals were represented by pyrite.*

Two specimens of granite were chosen in addition. We gathered specimen 1 at an absolute altitude of 3800 meters in the vicinity of the Tien-Shan Observatory of the Academy of Sciences USSR, and it represents the surface portion of

* The minerals were obtained from the Geological Museum of the Academy of Sciences, Kazakh SSR.

the rock. Specimen 1 is a heavily weathered, light gray, fine-grained granite with no lichen covering. Specimen 2 is a fresh granite with a feeble touch of weathering taken from falls of rock in the watershed region of the Terskey-Alatau Range above Ashuter Glacier (absolute height 4200 meters).

TABLE I. BULK ANALYSES OF ROCKS AND MINERALS USED FOR DECOMPOSITION
(IN PERCENTAGES OF THE FIRED MINERAL)

Order Number	Name of Object	Loss From Firing	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	P ₂ O ₅	CaO	MgO	K ₂ O	SO ₃
1	Granite	0.40	74.10	13.80	3.30	0.09	0.28	0.19	2.46	0.26
2	Orthoclase	0.17	69.50	17.00	none	none	none	none	9.22	none
3	Muscovite	1.30	45.30	39.66	2.44	.	.	6.83	5.47	.
4	Biotite	0.50	29.80	23.0	27.80	.	.	21.20	7.55	.
5	Serpentine	7.26	73.00	0.53	0.75	.	.	7.28	36.0	11.0
6	Lichen	—	—	—	—	15.00	—	—	—	—

Table I gives the bulk analyses of the minerals and rocks. Weighed portions of the minerals (from 0.5 to 5 grams), crushed fine and passed through a 0.25 mm mesh sieve, were placed in 100 cc Erlenmeyer flasks, flooded with 10 cc of distilled water or nutrient medium, and sterilized.

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The nutrient media were of the following composition (all media made with distilled water):

1. Complete nutrient medium: H₂O - 200 cc, K₃HPO₄ - 0.1 gram, MgCl₂ - 0.04 gram, CaCl₂ - 0.02 gram, (NH₄)₂SO₄ - 0.2 gram. Glucose - 4.0 gram.
2. Nutrient medium without potassium: H₂O - 200 cc, Na₂HPO₄ - 0.2 gram, MgCl₂ - 0.04 gram, CaCl₂ - 0.02 gram, (NH₄)₂SO₄ - 0.2 gram. Glucose - 4.0 gram.
3. Nutrient medium without phosphorus: H₂O - 100 cc, K₂SO₄ - 0.1 gram, MgCl₂ - 0.02 gram, (NH₄)₂SO₄ - 0.1 gram. Glucose - 4.0 gram.
4. Nutrient medium without magnesium: H₂O - 100 cc, K₂HPO₄ - 0.05 gram, CaCl₂ - 0.02 gram, (NH₄)₂SO₄ - 0.1 gram. Glucose - 2.0 gram.
5. Nutrient medium without sulfur: H₂O - 100 cc, K₂HPO₄ - 0.05 gram, CaCl₂ - 0.02 gram, (NH₄)₂NO₃ - 0.1 gram. Glucose - 2.0 gram.
6. Nutrient medium without magnesium, potassium, or calcium: H₂O - 100 cc, Na₂HPO₄ - 0.05 gram, (NH₄)₂SO₄ - 0.1 gram. Glucose - 2.0 gram.
7. Nutrient medium without potassium, magnesium, phosphorus, or calcium: H₂O - 100 cc, (NH₄)₂SO₄ - 0.01 gram. Glucose - 0.01 gram.
8. Nutrient medium with traces of C and N: H₂O - 200 g. Glucose - 0.01 gram, Peptone - 0.04 gram.

The weathered crusts of granite sterilely collected at an absolute altitude of 4250 meters in the Terskey-Alatau served as the inoculating material.

The surface portion of the crusts was scraped off with a sterile lancet, and 0.5 gram of finely crushed material was shaken up in 10 cc of sterile water. The mineral powders in the flasks suffused with nutrient media and sterilized were inoculated with two drops of the suspension obtained.

Two series of experiments were performed. The first series included all the above-listed minerals and the heavily weathered granite (specimen 1). The first series of experiments was laid out in the following pattern:

1. Mineral + distilled water was sterilized and left without inoculation to record the changes occurring in the sterile medium.
2. Mineral + incomplete nutrient medium without the elements present in the mineral; e.g., orthoclase + medium without potassium, apatite + medium without phosphorus, biotite + medium without potassium, magnesium, or calcium, and so on.
3. Aggregate of minerals + incomplete nutrient medium; e.g., muscovite, apatite, serpentine + medium without potassium, phosphorus, magnesium.
4. Granite + distilled water.
5. Granite + water with a small amount of organic carbon and organic nitrogen.
6. Granite + complete nutrient medium.

Except for the control flasks, all the others were inoculated with the suspension after addition of the nutrient media and sterilization.

The flasks, covered in black paper, stood at room temperature for six months (from 3 June 1948 to 5 January 1949). As the liquid dried up, distilled water was sterilely added to the minerals to bring the amount back to its previous volume.

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Every week microscopic observations of the appearance of microorganisms, their development, and the change in the appearance of the minerals were conducted. At the end of the experiment, smears were taken of the mineral deposit on the bottom of the flasks and of the liquid medium above the minerals.

The smears were fixed, stained with a phenol solution of erythrosin by the Vinogradskiy method, and examined under the microscope. Inoculations were made from all the flasks onto solid and liquid peptone-sugar media, potato-sugar media, and Capek's medium.

The contents of the flasks were evaporated until dry and successively treated with a 0.5 N solution of NaOH and a 0.05 N solution of HCl to extract the sesquioxides and silica freed during decomposition of the minerals.

In the hydrochloric filtrate, the SiO_2 was determined by the gravimetric method, Fe_2O_3 by the volumetric method and colorimetrically with sulfosalicylic acid, Al_2O_3 in the form of hydroxyquinolate by colorimetry, K_2O by the cobalt nitrite method, and CaO by precipitation with oxalic acid and subsequent volumetric determination.

In the alkaline extract SiO_2 (gravimetrically) and Al_2O_3 (colorimetrically) were determined.

Microscopic scrutiny of the mineral residues after treatment with acid and alkali disclosed incomplete solution of the skeletons of diatom algae in the 0.05 N alkaline solution. Therefore, the data given in Table III on the amorphous silica content are reduced.

The first series of experiments, moreover, had no control flasks with a complete nutrient medium (except for granite). We were therefore unable to account for microorganism growth under optimum conditions and in an incomplete medium + mineral.

A second series of the experiments was therefore set up in which granite (specimen 2) and the potassium-containing minerals orthoclase, muscovite, and biotite were used (the mineral portions weighed 1.0 - 0.5 gram).

The pattern was as follows:

1. Mineral + distilled water (the control).
2. Mineral + distilled water (inoculated).
3. Mineral + incomplete nutrient medium.
4. Mineral + complete nutrient medium.

In addition an investigation was made of whether microorganisms could utilize the organic matter and mineral elements of lichens. Our observations had shown that dust usually containing spores and fragments of lichen hyphae settled on the surface of naked rocks in the nival region. Parallel observations were also made of the mineral change and microorganism development under the conditions of:

1. Mineral + water with traces of carbon and nitrogen (glucose and peptone).
2. Mineral + water + bits of Gyrophora lichen.

After sterilization and subsequent inoculation, the second series of flasks stood at room temperature for three months (from 3 March to 10 June 1949). During this period, just as in the first series of experiments, development of the microorganisms was microscopically traced and at the end of the standing time smears for microscopic study and inoculations onto nutrient media were made.

Special preparations were made for studying the alterations which had occurred in the minerals themselves. The newly-formed secondary clayey minerals were accounted for by staining with preparations of malachite green and patent blue (by V. T. Belousova's method).

The air-dried residue in the flasks was treated with 100 cc of water. After filtration, the potassium in the filtrate was determined if it had not been introduced together with the nutrient medium. The residue on the filters was again flushed off into flasks with 5% NaOH and made into the usual alkaline extract with subsequent determination of the SiO_2 and Al_2O_3 .

Results of Investigations

In the first series of flasks, changes visible to the eye began to occur on the third day after inoculation. In some flasks the liquid above the minerals became turbid and in several concentric colonies of fungi (with a white mycelium) appeared. On the tenth day, considerable gelatinization of the minerals in the inoculated flasks, increased turbidity, and the formation of flocs had become perceptible. In the flasks with fungi the mycelium was covered with green spores. By the end of the second month, the microorganisms had attained their maximum development and thereafter no visible changes occurred in the flasks, except that in several flasks with abundant growth of fungi of the genus Penicillium gradually darkening was detected with continued standing, and was followed by acquisition of a brown color by the liquid and the mineral deposit. Table II gives the findings on the state of the minerals and the group composition of the microorganisms at the time the experimental readings were made (after six months).

No visible changes in the mineral deposit took place in the control flasks. Inoculations from these flasks onto nutrient media likewise failed to display microorganisms. The confident statement may thus be made that here the minerals changed only under the effect of the water and of the gases dissolved therein (oxygen, carbon dioxide).

In the media containing pyrite as the sulfur source, no visible changes in the minerals were discovered either, but the solution in the pyrite-containing flasks became slightly yellowish, although it remained transparent. Oxidation of the pyrite apparently generates large quantities of sulfuric acid, and this hinders microorganism growth.

A small number of bacterial cells was detected on the surface of the minerals in the media containing other minerals (orthoclase, apatite, serpentine) in addition to pyrite.

Rather abundant bacterial growth was found on granite specimens to which either no nutrient elements or traces of carbon and nitrogen had been added. A white bacterial slime covered the surface of the minerals, and the solution above them had also become turbid. Most of the mineral grains displayed coccoid bacterial agglomerations surrounded by slime after erythrosin staining of the preparations. The cells of this bacteria are arranged on the preparations without apparent order and form short chains only in spots. The size of the

TABLE IV

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Order Number	Name of Material	Composition of Medium	State of Material on Taking Experimental Readings	Microorganism Group Composition				
				Bacteria	Fungi	Diatom Algae	Green and Blue-Green Algae	
1	Granite	H ₂ O (uninoculated)	No Changes	-	-	-	-	
2	Granite	H ₂ O (inoculated)	White Bacterial Slime	++	-	-	+	
3	Granite	H ₂ O traces of C,N	Abundant White Bacterial Slime	++	-	-	+	
4	Granite	Complete Nutrient Medium	Tiny Well-Shaped Colonies of <u>Penicillium</u> Cover 60% of the Surface; Material is Gelatinized	++	+++	+	+	
5	Orthoclase	H ₂ O (uninoculated)	No Changes	-	-	-	-	
6	Orthoclase	Complete Nutrient Medium Without Potassium	Tiny colonies of <u>Penicillium</u> cover 90% of the surface; feeble white bacterial slime	++	+++	-	-	
7	Muscovite	H ₂ O (uninoculated)	No Changes	-	-	-	-	
8	Muscovite	Complete Nutrient Medium Without Potassium	Tiny <u>Penicillium</u> colonies cover 80% of the surface; material gelatinized and colored yellow	++	+++	+	-	
9	Biotite	H ₂ O (uninoculated)	No Changes	-	-	-	-	
10	Biotite	Complete Nutrient Medium Without Potassium	Tiny <u>Penicillium</u> colonies cover 70% of the surface; material gelatinized	+	+++	-	-	

TABLE IV

Order Number	Name of Material	Composition of Medium	State of Material on Taking Experimental Readings	Microorganism Group Composition				
				Bacteria	Fungi	Diatom Algae	Green and Blue-Green Algae	
11	Apatite	H ₂ O (uninoculated)	No Changes	-	-	-	-	
12	Apatite	Complete Nutrient Medium Without Phosphorus	Tiny dark-green <u>Penicillium</u> colonies and abundant bacterial slime; yellow coloration	++	+++	.	+	
13	Serpentine	H ₂ O (uninoculated)	No Changes	-	-	-	-	
14	Serpentine	Complete Nutrient Medium Without Magnesium	Large <u>Penicillium</u> colonies cover 90% of the surface; material gelatinized and colored yellow	+++	+++	+	+	
15	Orthoclase Apatite Serpentine	H ₂ O traces of C,N,S	White Bacterial Slime Sparse	++	-	+	+	
16	Orthoclase Apatite Serpentine Pyrite	H ₂ O traces of C,N	No Changes	+	-	-	-	
17	Muscovite Apatite Serpentine	H ₂ O traces of C,N,S	Abundant White bacterial slime	+++	-	+	+	
18	Muscovite Apatite Serpentine Pyrite	H ₂ O traces of C,N	No Changes	+	-	-	-	
19	Pyrite	Complete Nutrient Medium Without Sulfur	No changes; solution colored yellow	-	-	-	-	

TABLE IV

Order Number	Name of Material	Composition of Medium	State of Material on Taking Experimental Readings	Microorganism Group Composition				
				Bacteria	Fungi	Diatom Algae	Green and Blue-Green Algae	
20	Pyrite	Complete Nutrient Medium	No changes; solution colored yellowish	-		-		-

Note:—Denotes no microorganisms; + few; ++ fairly many; +++, very many.

bacteria is 1.2 - 1.3 microns. Here, too, was detected a small number of diatom and green algae. Among diatom algae we find Pinnularia, while green algae are represented by single-celled forms of the genus Chlorococcum.

Fungi of the genus Penicillium developed in abundance on the granite with the complete nutrient medium. In solution and on the surface of the minerals, many coccoid bacteria and a small number of diatom and green algae are detected.

Just as abundant a growth of Penicillium and the coccoid bacterium was observed on all incomplete nutrient media containing an adequate amount of organic carbon and organic nitrogen, with the missing elements added in the form of minerals, as was seen on the granite with the complete nutrient medium. This indicates that it is possible for microorganisms to extract the missing elements of nutriment from the minerals. /85

No fungi grew on incomplete nutriment media with traces of carbon and nitrogen and with addition of certain minerals as the source of nutriment, but everywhere there was an appreciable development of coccoid bacteria and diatom and green algae.

On a solid peptone-sugar agar medium, the bacteria formed thick whitish colonies with clearly drawn edges.

Two species of Penicillium were discovered in subinoculations onto a solid peptone-agar medium. One of them did not customarily color the nutrient medium, or did so only weakly; the second strongly colored the medium straw-yellow and brown. This fungus grew on muscovite, serpentine, and apatite, and strongly colored the liquid in the flasks and the mineral deposit. No actinomycetes were discovered in any of the flasks.

Table III gives the analyses of the weakly acid and weakly alkaline extracts from the minerals subjected to microorganism action.

TABLE III. RESULTS FROM 0.05 N HCl EXTRACTS SUBSEQUENTLY TREATED WITH 0.05 N NaOH (IN PERCENTAGES OF BULK CONTENT)

Order Number	Name of Object	K ₂ O	CaO	Fe ₂ O ₃	Al ₂ O ₃	SiO ₂	State of Flasks at End of the Experiment
1	Granite + H ₂ O (control)	2.35	0.06	2.18	0.04	0.31	No Changes
2	Granite + H ₂ O (inoculated)	9.60	0.31	12.70	0.33	0.76	White bacterial slime
3	Granite + H ₂ O + C + N	15.20	0.27	10.90	0.73	0.77	Abundant white bacterial slime
4	Granite + Complete Nutrient Medium	Not Determined	Not Determined	7.28	0.123	0.76	Abundant Penicillium colonies and bacterial slime
5	Orthoclase + H ₂ O (control)	0.82	"	None	None	0.08	No Changes
6	Orthoclase + Complete nutrient without potassium	1.80	"	None	Traces	0.41	Penicillium colonies and bacterial slime
7	Muscovite + H ₂ O (control)	1.05	"	None	None	0.12	No Changes
8	Muscovite + Complete nutrient medium without potassium	2.35	"	None	Traces	0.85	Abundant Penicillium colonies and bacterial slime
9	Biotite + H ₂ O (control)	0.98	None	Traces	Traces	0.24	No Changes
10	Biotite + Medium Without potassium	2.65	0.04	0.54	Traces	0.97	Penicillium colonies and bacterial slime
11	Serpentine + H ₂ O (control)	Not Determined	Not Determined	Traces	None	0.22	No Changes
12	Serpentine + Complete nutrient medium without magnesium	"	"	9.60	None	0.70	Abundant Penicillium colonies and bacterial slime

TABLE IV

Order Number	Name of Material	Medium Number	Composition of Medium	State of Material on Termination of Experiment	Microorganism Group Composition					
					Bacteria	Fungl	Diatom	Algae	Green & Blue	Algae
1	Granite (control)	----	H ₂ O	No Changes	-	-	-	-	-	-
2	Granite	----	H ₂ O	No Changes	+	-	+	+	-	-
3	Granite	----	H ₂ O + algae	Weak gelatinization	+	-	+	+	-	-
4	Granite	----	H ₂ O + gyrophora	Perceptible gelatinization	+	-	+	+	-	-
5	Granite	5	H ₂ O, traces of C,N	Perceptible gelatinization	+++	-	+	+	-	-
6	Granite	1	H ₂ O, P, S, K, C,N	Surface covered with colonies of Penicillium	++	++	++	++	++	-
7	Orthoclase (control)	----	H ₂ O	No Changes	-	-	-	-	-	-
8	Orthoclase	----	H ₂ O	No Changes	+	-	-	-	-	+
9	Orthoclase	2	H ₂ O, P, S	Perceptible gelatinization	++	-	-	+	+	+
10	Orthoclase	2.5	H ₂ O, P, S, traces C,N	Heavy gelatinization	+	-	-	-	-	-
11	Orthoclase	3	H ₂ O, P, S, C, N	Penicillium colonies cover 50% of surface	+	+	+	+	+	-
12	Orthoclase	1	P, S, K, C, N	Penicillium colonies cover 60% of surface	++	++	++	++	++	-
13	Muscovite (control)	----	H ₂ O	No Changes	-	-	-	-	-	-
14	Muscovite	----	H ₂ O	No Changes	+	+	+	+	+	-

TABLE IV

Order Number	Name of Material	Medium Number	Composition of Medium	State of Material on Termination of Experiment	Microorganism Group Composition					
					Bacteria	Fungus	Algae	Green	Blue	Green Algae
15	Muscovite	2	H ₂ O, P, S,	Heavy gelatinization	++	-	-	-	-	-
16	Muscovite	2.5	H ₂ O, P, S, traces C, N	Yellow coloration, <u>Penicillium</u> colonies	++	+++	+	+	+	++
17	Muscovite	3	H ₂ O, P, S, C, N	Yellow coloration, <u>Penicillium</u> colonies	++	+++	+	+	+	++
18	Muscovite	1	H ₂ O, P, S, K, C, N	Yellow coloration, <u>Penicillium</u> colonies	++	+++	+	+	+	++
19	Biotite (control)	----	H ₂ O	No Changes	-	-	-	-	-	-
20	Biotite	----	H ₂ O	Weak gelatinization	+	+	-	-	+	+
21	Biotite	2	H ₂ O, P, S,	Heavy gelatinization	+++	-	-	-	-	-
22	Biotite	2.5	H ₂ O, P, S, traces C, N	<u>Penicillium</u> colonies	++	+	-	-	-	-
23	Biotite	3	H ₂ O, P, S, C, N	Abundant <u>Penicillium</u> Colonies	++	+++	-	-	-	-
24	Biotite	1	H ₂ O, P, S, K, C, N	Abundant <u>Penicillium</u> Colonies	++	+++	-	-	-	-

Note: --- None, + Few, ++ Fairly Many, +++ Very Many

These minerals so subjected are considerably more soluble in weak acid and alkali than the minerals in a sterile medium.

Potassium solubility is particularly high. Orthoclase, muscovite, and biotite yielded approximately twice as much soluble potassium as did the control. An especially large amount of mobile potassium was discovered in the granite specimens inoculated with microorganisms (four to six times more than in the control). Mobility of iron is also greatly elevated, i.e., five to six times above the control in the case of the granite specimens. Ten percent of the bulk iron content was dissolved in serpentine and 0.5% in biotite.

Silica solubility in 0.05 N NaOH is low. Microscopic investigations indicated that the skeletons of the diatom algae remained undissolved. It is also possible that the secondary clayey minerals containing SiO_2 and Al_2O_3 did not undergo dissolution in this treatment, but nevertheless in all cases a rise in soluble SiO_2 by a factor of two to five times over the controls was evident in the inoculated flasks. Aluminum oxides were ascertained in very small quantities, and only in granite specimens.

The second series of experiments lasted for three months. Microorganism growth, as in the first case, began on the third or fourth day after inoculation. The microorganisms had reached their maximum development by the end of the first month. Further changes took place only in several flasks with an abundant growth of Penicillium. The solution became yellow-brown and the mineral deposit took on a cinnamon brown color.

Table IV presents the results of the microscopic observations and the microscopic investigation of the group composition of the microorganisms three months after the beginning of the experiment.

As in the first series of experiments, the control flasks were sterile. No changes perceptible to the eye occurred in the flasks containing mineral + water and inoculated with microorganisms. Microscopic investigation revealed a very small amount of bacterial slime and isolated bacterial cells on the surface of the minerals. In several cases (granite, orthoclase, muscovite) diatom algae of the genera Navicula, Eunotia, Cocconeis, and Meriodion grew on this surface.

When bits of Gyrophora lichen were added to the mineral to act as the source of organic and mineral substances, observation showed appreciable gelatinization and rather abundant development of tiny coccoid bacteria both on the surface of the mineral particles and in the solution. Here, too, in most cases diatom algae were present. A great number of tiny coccoid bacteria (1.2-1.4 microns in diameter) grew on the minerals with an incomplete nutrient medium having traces of carbon and nitrogen, as was also the case in the first series of experiments. The colonies of these coccoid bacteria were surrounded with slime and covered a large part of the mineral surface, also being encountered in substantial quantity in the solution. Here, too, developed blue-green, green, and diatom algae (Figure 1, 3a, c).

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TABLE V. RESULT OF SECONDARY NEW-FORMATION COUNT

Name of Object	Name of Micro-Organism	FOUND IN 10 VISUAL FIELDS			
		Particles Stained by Malachite Green	Calcite Grains	Skeletons of Diatom Algae	Grains of Amorphous Silica
Granite + H ₂ O (Control)	Sterile	17	4	----	----
Granite + H ₂ O (Inoculated)	Bacteria	93	35	3	----
Granite + H ₂ O + Gyrophora	Bacteria	84	40	6	5
Granite + H ₂ O + C + N	Bacteria	60	47	3	7
Granite + Complete Nutrient Medium		346	62	7	31
Orthoclase + H ₂ O (Control)	Sterile	8	----	----	----
Orthoclase + H ₂ O (Inoculated)	Bacteria	19	----	----	----
Orthoclase + H ₂ O + Gyrophora	Bacteria	34	5	1	3
Orthoclase + Nutrient Medium Without Potassium	Fungi	43	1	----	4
Orthoclase + Complete Nutrient Medium	Fungi	42	22	----	3

When an incomplete nutrient medium with a normal content of carbon and nitrogen was added to the minerals, fungi of the genus Penicillium grew alongside the bacteria.

Fungal growth intensity and character were identical in the flasks with incomplete nutrient medium + mineral and in those with the complete nutrient medium. In each case fungus colonies covered 80-90% of the bottom surface of the flask and sporulated well (Figure 1).

Fungi grew with especial luxuriance on muscovite, biotite, and granite. There was somewhat weaker fungal growth on orthoclase.

The minerals, particularly platelets of muscovite and biotite, were partially enmeshed in a solid web of fungal hyphae and floated together with the mycelium on the surface of the liquid. A large part of the mineral granules on the bottom of the flasks also bore fungal hyphae on their surface. The surface of some granules -- particularly noticeably on biotite lamellae -- was greatly corroded. Just as in the first series of experiments, the development of Penicillium provoked yellow coloring in the liquid and minerals, particularly strongly in the flasks containing muscovite. A large part of the muscovite lamellae was covered with a yellow-brown, opaque, slightly granular substance.

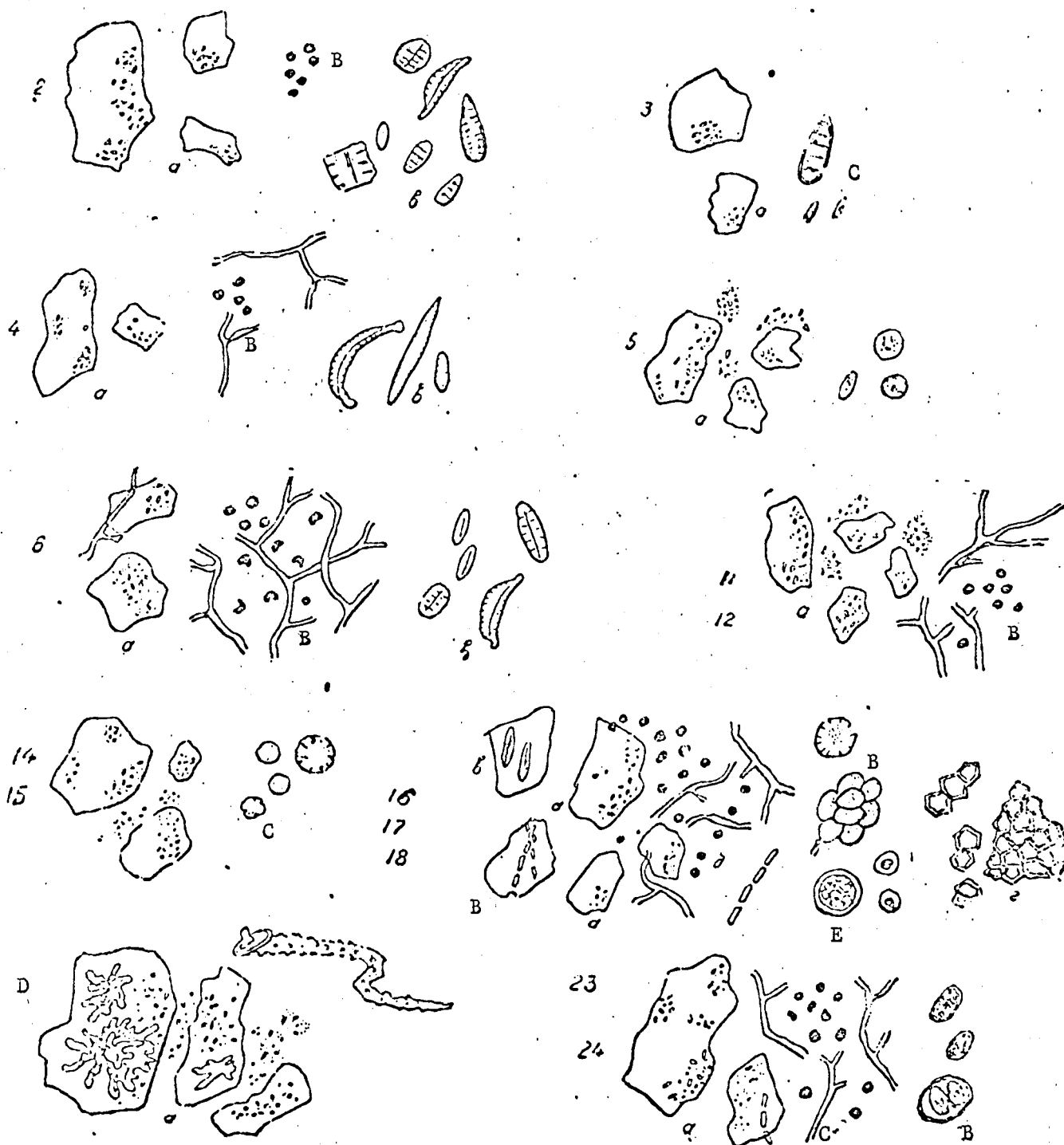
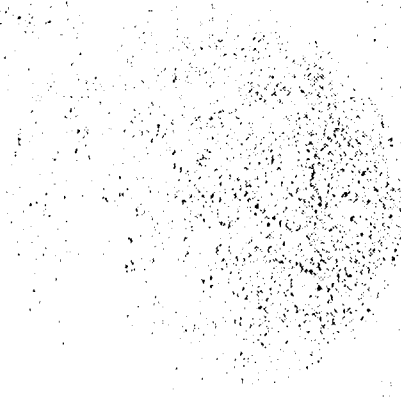
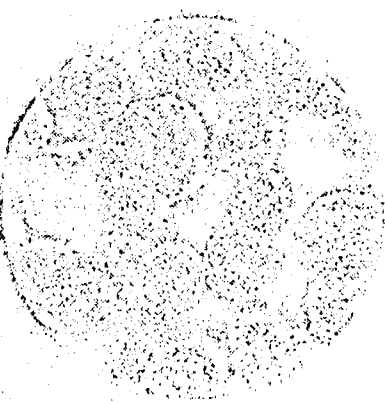


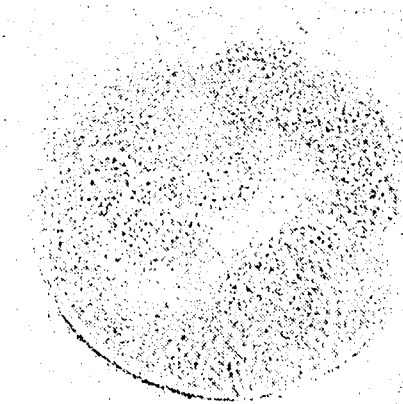
Figure 1. Microflora Grown on Minerals Inoculated with Products of Granite Weathering. (a) Coccioid Bacteria, (b) Fungal Spores and Hyphae, (c) Diatom Algae, (d) Colonies of Silicon Skeleta of Protozoans, (e) Green and Blue-Green Algae, 2,3 etc., Flask Numbers.



Orthoclase Control



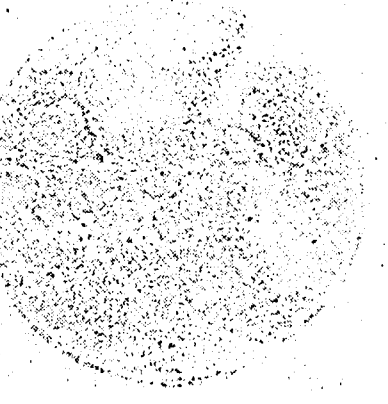
Orthoclase + Medium
Without Potassium



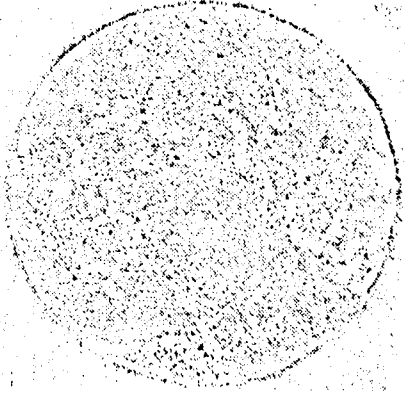
Orthoclase + Complete
Nutrient Medium



Muscovite Control



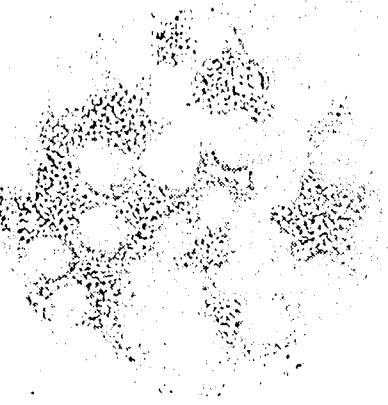
Muscovite + Medium
Without Potassium



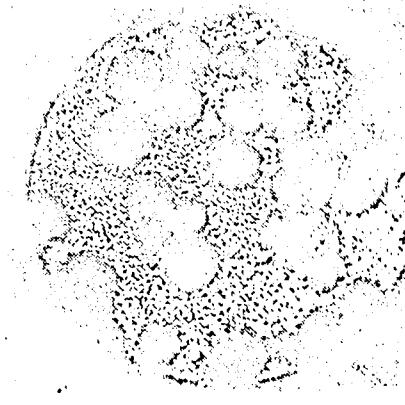
Muscovite + Complete
Nutrient Medium



Biotite Control



Biotite + Medium
Without Potassium



Biotite + Complete
Nutrient Medium

Figure 2.

Development of Penicillium on Minerals

When examined under the microscope, the mineral residues displayed the formation of small granules and aggregations of calcite in the flasks containing calcium either in the mineral composition or in the nutrient medium. The more abundant the microorganism growth, the greater was the amount of calcite. A count of the calcite grains under a microscope showed that they formed most of all on granite populated with bacteria, and particularly with fungi (Table V). ^{/89} A significant quantity of calcite was discovered on products of biotite weathering (dolomite, which is close to calcite in its optical properties, may also have partially been formed there).

In the muscovite and orthoclase-containing flasks there was less calcite and it showed up only in cases where the nutrient medium contained calcium. The calcite grains were for the most part tint (0.005 to 0.01 mm in diameter), irregular in shape, and with evident cleavage only in the large examples.

A malachite-green stain was used on the preparations with mineral particles in order to determine other secondary new formations.

The malachite-green stained the individual grains in the flasks not inoculated with microorganisms. The number of stained grains increased greatly if the minerals had been populated by bacteria, or fungi in particular. Table V gives the results of counting the stained grains in ten fields of vision (with a uniform distribution of 0.001 gram of mineral deposit over an area of one square centimeter).

A particularly large number of secondary minerals stained with malachite-green was detected in the products of granite breakdown (20 times more of them on granite with Penicillium than in the control flask). Orthoclase decomposes to a considerably less degree (there are only five times more clayey minerals on the orthoclase with Penicillium than in the control flask).

The indices of refraction for the grains stained blue-green in the granite specimens fluctuated from 1.510 to 1.543, which corresponds to beidellite or montmorillonite. The blue-green grains on orthoclase read 1.549 - 1.556, which corresponds to beidellite. In one instance, grains of a green color with $n = 1.560$ were encountered -- apparently sericite.*

In the products of granite and orthoclase weathering, moreover, irregularly shaped amorphous grains of low refractive index (1.48) and blue staining were discovered. These are apparently amorphous silica. Some diatom algae skeletons also stained blue. Table V gives the count of diatom skeletons and grains of amorphous silica in the weathering products of granite and orthoclase. On the granite specimens particularly populated by fungi, a substantially larger number of diatom skeletons and of amorphous silica was found than in orthoclase weathering products.

Staining of the muscovite preparations demonstrated that this mineral had been subjected to very severe decomposition. A substantial portion of the

*Diagnosis of clayey minerals by optical methods and staining cannot be considered absolutely accurate (Author's note).

muscovite lamellae in the flasks with bacterial microflora was partially stained greenish-blue. The index of refraction could be determined on individual tiny flakes and was approximately 1.510. On the specimens with fungal microflora, the muscovite was entirely stained a greenish-brown by the malachite-green. It was difficult to ascertain the refractive index of the secondary new formations on the muscovite, since a large part of the surface was covered with an opaque brownish organic substance. The products of muscovite weathering revealed a large number of Pinnularia diatom algae, forming whole colonies in places (Figure 1, Nos. 16, 17, 18c). Flasks 16 and 17 (muscovite and Penicillium) contained the silicon cysts of protozoans in addition to diatom algae (Figure 1, Nos. 16, 17, 18). Here were also found grains of amorphous silica and tiny crystals of calcite.

The products of biotite weathering contain no secondary new formations at all except rather frequent calcite grains, coarse and with well-marked cleavage. An elongated amorphous body with a low refractive index close to that of opal /90 was likewise found here. The surface of the biotite lamellae very often had a greatly eroded appearance, especially at the sites of fungal hyphae accumulations (Figure 1, No. 21a).

Table VI presents data on aqueous and alkaline extracts from weathering products of granite, orthoclase, muscovite, and biotite. Only potassium was found in the aqueous extracts. The granite specimens exposed to the action of coccoid bacteria (Flasks 2, 3, 4) exhibited a significantly larger quantity of water-soluble potassium than did the granite specimen situated in sterile circumstances (Flask 1).

The biotite weathering products exhibited the same pattern.

In the orthoclase and muscovite specimens the quantity of water-soluble potassium is approximately the same in the sterile flasks as it is in those with fungal and bacterial microflora. The slight amount of water-soluble potassium in the flasks with abundant fungal and bacterial microflora may be explained by the fact that a substantial part of the potassium liberated in decomposition of the minerals is situated in the microorganism bodies and does not pass into the aqueous extract.

The degree of breakdown of aluminosilicates may be judged from the quantity of SiO_2 and Al_2O_3 passing into an alkaline extract. To be sure, we cannot assert that all secondary clayey minerals dissolve in 5% of alkali and that the figures obtained represent the true quantity of silicon and aluminum oxides liberated during weathering, but the figures quoted are indicative enough of the comparative characteristic tendency of the individual minerals and rocks to weather. A comparison of the concentrations of SiO_2 and Al_2O_3 dissolved in alkalies in the control flasks and in those inoculated with microorganisms shows that in /92 all cases the control flasks contain substantially fewer oxides soluble in alkalies than do the flasks with bacterial, and particularly fungal, microflora. The muscovite settled by fungi decomposed most heavily. Under conditions of an incomplete nutrient medium where it replaced the insufficient potassium (flask 14) 9.2% of the bulk content of SiO_2 and 8.5% of the bulk content of Al_2O_3 passed into the alkaline extract.

TABLE VI. RESULTS FROM AQUEOUS AND ALKALINE EXTRACTS
(IN PERCENTAGE OF BULK CONTENT)

Order Number	Name of Object	Aqueous Extract			5% KOH Extract			Flask Condition At Termination of Experiment
		K ₂ O	SiO ₂	Al ₂ O ₃				
1	Granite + H ₂ O (control)	0.68	0.58	None				No microorganisms
2	Granite + H ₂ O (inoculated)	8.85	0.99	1.02				Slight gelatinization
3	Granite + H ₂ O + Gyrophora	23.7	0.85	0.51				Appreciable gelatinization
4	Granite + H ₂ O + C + N	8.15	1.08	0.77				Appreciable gelatinization
5	Granite + Complete Nutrient Medium	Not Determined	2.44	3.08				<u>Penicillium</u> colonies
6	Orthoclase + H ₂ O (control)	0.94	0.77	0.19				No microorganisms
7	Orthoclase + H ₂ O (inoculated)	0.94	0.87	0.19				No perceptible changes
8	Orthoclase + H ₂ O + Gyrophora	1.08	1.06	0.59				Gelatinization
9	Orthoclase + Nutrient Medium Without Potassium	0.96	0.90	0.19				Gelatinization
10	Orthoclase + Complete Nutrient Medium	Not Determined	1.11	0.98				<u>Penicillium</u> colonies
11	Muscovite + H ₂ O (control)	1.26	2.27	0.34				No microorganisms
12	Muscovite + H ₂ O (inoculated)	0.85	3.18	1.29				Gelatinization
13	Muscovite + H ₂ O + Gyrophora	0.63	2.89	0.67				Gelatinization
14	Muscovite + Nutrient Medium Without Potassium	1.57	9.25	8.55				<u>Penicillium</u> colonies
15	Muscovite + Complete Nutrient Medium	Not Determined	6.64	5.58				<u>Penicillium</u> colonies

TABLE VI. RESULTS FROM AQUEOUS AND ALKALINE EXTRACTS
(IN PERCENTAGE OF BULK CONTENT)

Order Number	Name of Object	Aqueous Extract			Flask Condition At Termination of Experiment
		K ₂ O	SiO ₂	Al ₂ O ₃	
16	Biotite + H ₂ O (control)	1.13	2.43	0.29	No microorganisms
17	Biotite + H ₂ O (inoculated)	1.67	3.10	None	Weak gelatinization
18	Biotite + H ₂ O + Gyrophora	2.25	3.77	0.58	Gelatinization
19	Biotite + Complete Nutrient Medium Without Potassium	2.25	3.52	0.35	<u>Penicillium colonies</u>
20	Biotite + Complete Nutrient Medium	Not Deter- mined	5.55	2.33	<u>Penicillium colonies</u>

TABLE VII CHANGE IN pH OF MEDIUM FROM EFFECT OF TEN-DAY GROWTH OF
MICROORGANISMS EVOLVED IN DECOMPOSITION OF MINERALS

Name of Rock or Mineral on Which Micro- organism developed	General Aspect of Culture and Name of Microorganism	Color of Solution	pH of Potato- Sugar Medium		pH of Yeast Wort	
			Before Experiment	After Experiment	Before Experiment	After Experiment
Granite	<u>Penicillium</u> , unbroken film of liquid surface in state of sporulation (green spores)	Lemon yellow	7.05	3.50	6.50	3.50
Muscovite	Unbroken film of white <u>Penicillium mycelium</u> , rarely sporulates	Brown	7.05	4.75	7.70	3.80
Apatite	White mycelium of <u>Peni- cillium</u> forms film on surface	Lemon yellow	7.05	5.30	6.30	4.70
Biotite	Unbroken film of <u>Peni- cillium</u> on surface in state of sporulation	Brown	7.05	4.80	6.70	3.88
Granite	Flocs of white bacterial slime in abundance	Unchanged in Com- parison with ini- tial medium	7.05	6.70	----	----
Orthoclase	Flocs of white bacterial slime in abundance	Unchanged	7.05	7.50	----	----
Control	No Changes	Unchanged	7.05	6.95	----	----

Under conditions of a complete nutrient medium (Flask 15), the quantity of SiO_2 and Al_2O_3 passing into the extract is somewhat less, but nevertheless substantial.

Granite stands in second place in regard to degree of solubility of SiO_2 and Al_2O_3 . As on muscovite, the development of bacteria favors the liberation of SiO_2 and Al_2O_3 to a lesser degree than does the growth of fungi. In the last case, the appreciable quantities of aluminum oxides soluble in alkalies (3.08% of bulk content) indicate the breakdown of aluminosilicates. The figure for the silica dissolved in alkalies is significantly reduced, because it is computed from the bulk content of SiO_2 in the granite without subtracting the silica of the quartz.

The decomposition of biotite under the action of microorganisms, particularly when fungi are developed, is also rather appreciable. Orthoclase is least of all subject to microorganism action; its degree of solubility in alkalies rises little in the inoculated flasks over the control.

The earlier presented results of microscopic investigation of secondary new formations on diverse minerals are in good agreement with the findings of the aqueous and alkaline extracts.

The Role of Fungi in Mineral Decomposition Processes and in Formation of Organic Matter

The series of experiments cited have indicated that penecillia inhabiting the weathering products of granite exert the greatest decomposing effect on minerals. The coccoid bacteria living together with them break down minerals to a lesser degree.

To clarify the mechanism of the action of fungi on minerals, we made observations of the change in pH of nutrient media after the effect of microorganisms grown on minerals. The media used for the experiment were non-buffer and contained a large amount of bases, potato-sugar and yeast wort, to be precise.

Table VII lists the results of electrometric tests of pH before and after ten days of microorganism growth.

It was found that in a bacterial culture the pH remains unchanged or even rises somewhat -- the medium becomes slightly alkalized. Penicillium, on the contrary, which is liberated by various minerals drastically acidifies the medium in its first stages of growth and reduces the pH in some cases to 3.5-3.8. During growth of the fungi, the color of the nutrient media changed to lemon yellow and brown.

The yellow and brown hue is also seen to appear during growth of fungi on minerals. Several aqueous extracts, the mineral deposit, and, in particular, the

alkaline extracts were tinted from light yellow to dark yellow (the color of strong tea).

We attempted to clarify the nature of the colored acids excreted by Penicillium fungi during growth. For this purpose, the total carbon content of the stained aqueous and alkaline extracts was determined and the organic matter was fractionally analyzed by the method of I. V. Tyurin. The humic and fulvic acid fractions were isolated and their carbon content was ascertained (we were unsuccessful in determining nitrogen because of the small quantities of solution). The carbon in the mineral residue after treatment was also determined. Table VIII gives the analytical results.

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The aqueous extracts were colorless in most cases. In the extracts stained straw-yellow, a small quantity (2.6-4.4%) of the originally introduced carbon was detected, but the most highly colored aqueous extract from a muscovite specimen contained 10% of the originally introduced carbon. The bulk of the newly-formed organic substances passed over into the subsequent alkaline extract. All the alkaline extracts from minerals with fungal microflora were stained from yellow to brown in color. In subsequent neutralization and addition of sodium sulfate, almost all cases saw the precipitation of a dark-brown flocculent residue resembling that of humic acids. Like the latter it is completely soluble in alkalis. A determination of the carbon in an alkaline solution of humic acids showed that this fraction contains from 1.0 to 3.7% of the carbon introduced when the experiments were set up. In only one case in the granite specimen was the humic acid fraction lacking.

After precipitation of the humic acid, the filtrate containing the fulvic acid fraction -- usually lighter in color than the initial alkaline solution -- was boiled down and the carbon therein was also determined. The carbon concentration in this fraction fluctuates considerably in the different specimens. The greatest quantity of fulvic acid was isolated from the flasks with weathered muscovite (26-27% of the carbon that was introduced at the beginning of the experiment). In specimen 6 (muscovite + complete nutrient medium) only 15% of the carbon was detected in the fulvic acid fraction in the alkaline extract, but the preceding aqueous extract from this specimen had been an intense yellow and 10.6% of the carbon had been found in it. It is evident that here part of the fulvic acids had dissolved in the water. The total of the fulvic acid carbon in aqueous and alkaline solutions here is about 26% and is close to the rest of the figures for fulvic acids evolved from muscovite specimens.

A somewhat smaller, but still substantial, quantity of fulvic acids passed into the alkaline extract from specimens of biotite and granite (15-22%). A very small amount of fulvic acid (2.8-4.4% of total carbon) was extracted from specimens of weathered orthoclase; the alkaline extract here was very weakly colored.

A comparison of the findings on fulvic acid concentration in products of mineral weathering (Table VIII) with the amounts of SiO_2 and Al_2O_3 passing into the alkaline solution (Table VI), as well as with the results of calculating the secondary new-formations, shows that the degree of decomposition of the minerals

is directly dependent on the quantity of fulvic acids formed during development of the fungal microflora.

We noted above that Penicillium grown on mineral weathering products strongly acidifies the medium. The pH values which we found (3.5-3-6) are close to the same ones derived by V. V. Ponomareva for fulvic acids subjected to electro-dialysis.

Many investigators have studied the action of organic acids extracted from peat, humus, or duff on various minerals (experiments by Meshcherskiy, Niklas, Nikiforov; we cite after K. D. Glinka, 1931).

In some cases, e.g., in Meshcherskiy's research, a significant solvent capacity was discovered in the organic acids of soil humus. In other cases, as, for example, in the studies by Nikiforov and in the experiments of Remezov and his collaborators, minerals treated with aqueous extracts from duff were not observed to dissolve.

In all these cases the investigators did not operate with free organic acids, but with their salts derived from reaction with the mineral portion of soils or with the ash elements of the duff. V. R. Vil'yams in studying podzolizing processes in soils pointed out the breakdown of minerals under the effect of crenic acids excreted by fungi. Recently this position of V. R. Vil'yams has been confirmed by the experiments of V. V. Ponomareva (1947) who treated minerals with free electro-dialyzed fulvic acids extracted from podzolic soils. In this case it was found that they had substantial solvent capability.

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In our experiments, the reaction of aqueous extracts from specimens of weathered minerals in every case approached a neutral reaction, and was often weakly alkaline, while in a culture of fungi on nutrient media not containing minerals and poor in bases the reaction became strongly acid.

The organic acids excreted by fungi (perhaps not only the fulvic acids, but a number of others) acted directly and efficiently on minerals and produced new organomineral complexes, and in the meantime the medium remained neutral.

The quantity of carbon bound in humic and fulvic acids (extracted alkali) in our experiments fluctuated from 5.4 to 30.7% of the carbon introduced. If we figure out this quantity of carbon in percentages of the weight of the mineral residue, it amounts to 0.43 to 2.35% or from 0.7 to 4.0% of the humus. The carbon found in mineral residues after their treatment with alkali was represented in part by a brown organic substance imparting color to the minerals, and to a considerably greater measure by bodies of fungi and bacteria (it must be noted that the fungal and bacterial spores were also present in the last two fractions).

In all cases, the sum of all the carbon found in the aqueous and alkaline extracts and in the residue was less than 50% of the carbon introduced.

A substantial part of the carbon was volatilized in the form of CO_2 in the respiration process of the microorganisms. Part of the carbon combined with bases in the minerals and produced the alkali carbonates discovered in the aqueous extracts of mineral weathering products by their reaction with phenolphthalein. The newly formed alkaline-earth carbonates were, as already noted above, discovered in the mineral residue in the form of numerous tiny crystals and agglomerations of calcite.

Participation of Green and Blue-Green Algae in Calcite Formation

In most of the flasks of minerals inoculated with granite weathering products in our experiments, there were single-celled green algae of the genus Chlorococcum and filamentous forms of blue-green algae.

In order to verify whether algae always are present on the surface of weathered rocks in the nival region, we placed bits of weathered granites, limestones, and diorites in a ten-times diluted Heitler nutrient medium. Abundant algae colonies grew on all the inoculated specimens.

In a microscopic examination of the algae, numerous and variegated aggregations of calcite were found to be included inside the colonies. Many of the aggregations had the form of oolites connected in twos or in while groups (Figure 3, No. 2). Radially rayed concretions consisting of tiny crystals overlapping each other like scales were encountered just as often (Figure 3, No. 2); when crushed they fell into four or more wedge-shaped agglomerations (Figure 3, No. 2a).

In some cases the calcite was excreted in the form of concretions seemingly /97 like a shell consisting of large crystals or in the form of symmetrically arranged scale agglomerations with a bridge in the middle (Figure 3, No. 3). The calcite concretions often occupied more than 50% of the area of the preparations having algae colonies. Sometimes in their shell form they surrounded filaments of green-blue algae (Figure 3, No. 4).

The colonies of green and, in particular, of blue-green algae were almost always surrounded by bacterial slime, in the midst of which could be distinguished cells of coccoid bacteria. When the individual calcite concretions isolated from the remaining mass were dissolved in hydrochloric acid and when staining was performed with preparations with erythrosin, a large number of coccoid bacteria, apparently included within the calcite agglomerations, were also discovered.

Thus, in solutions containing calcium (Heitler's medium) the development of green and blue-green algae and their concomitant bacteria is accompanied by the massive formation of calcite.

Calcite formation does not proceed only in media containing calcium salts in solution. In a number of our experiments with granite and biotite, calcium and magnesium were present only as minerals, but nevertheless calcite formation was everywhere observed, although in lesser quantities than on Heitler's medium.

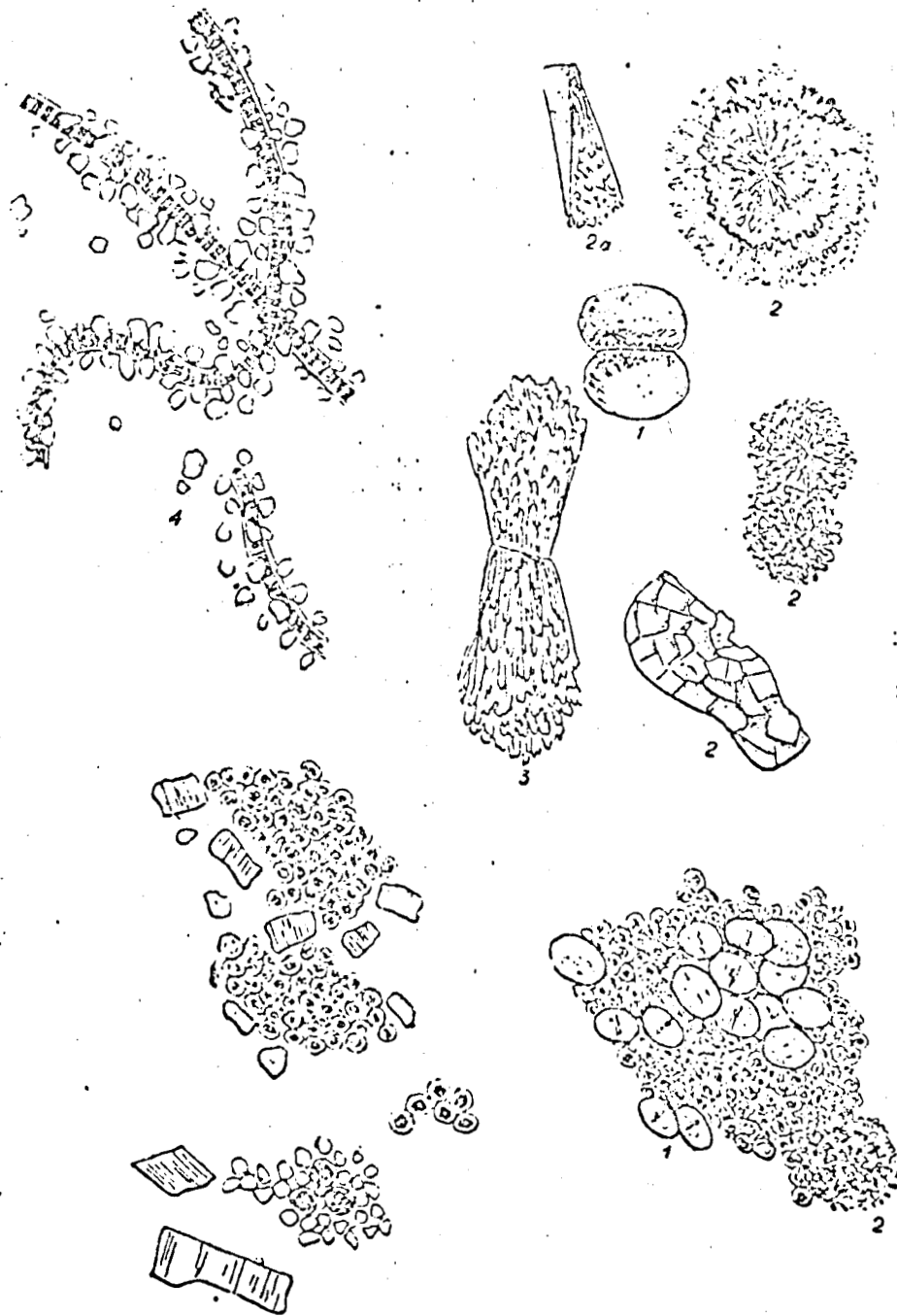


Figure 3. New Formations of Calcite Amid Green and Blue-Green Algae.

The results of our mineralogical investigations of the weathering crusts forming on the surface of rocks in the nival region completely coincide with laboratory experiments. The massive accumulation of calcite in products of rock weathering is usually confined to colonies of green and blue-green algae. Filaments of blue-green algae are often enclosed as though in a case of tiny calcite crystals (Figure 3, No. 4).

The question of the agent which plays the principal role in calcite formation -- the algae or the bacteria living together with them or both organisms in equal degree -- remains open.

It is known that many algae inhabiting basins of water are capable of precipitating lime.

In the process of photosynthesis, they absorb carbon dioxide dissolved in water and create conditions for the transition of bicarbonates into the less soluble carbonates. The surface of algae and the stones on which they live on the bottom of basins of water are often continuously incrustated with tiny crystals of calcite.

The role of bacteria in calcite formation has also been repeatedly discussed in the works of microbiologists. The research of Nadson, Drew, Molisch, Brussov, and others has established the fact that calcite is formed in cultures of several bacteria. The forms of the calcite deposits in Nadson's experiments are very similar to those obtained by us.

B. L. Isachenko (1948), while studying the thermal springs in Pyatigorsk and the water of Lake Sevan, discovered bacteria which deposit calcium carbonate from solutions. In laboratory experiments, B. L. Isachenko observed gradual crystallization of the colloid precipitate of calcium carbonate and the formation of spherulites and oolites of calcium including bacterial bodies.

The findings of H itler (we quote after B. L. Isachenko) indicate that deposition of CaCO_3 on Rivulariaceae algae occurs in the slime formed by the bacteria on their surface. This also coincides with Korinek's observations, who segregated calcite-forming bacteria from the surface of blue-green algae.

N. A. Krasil'nikov (1949) discovered in the rhizosphere of the higher plants bacteria which are able to precipitate calcium carbonate. The massive overgrowth of rocks included in soil layers by calcium carbonate is due to the action of these bacteria.

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B. L. Isachenko (1948) advances the hypothesis that adsorption of CaCO_3 is the source not only of carbon for heterotrophic bacteria, but also of energy which is released in the passage of colloid CaCO_3 into the crystal state. He writes that, just as green plants bind carbon dioxide into organic substances, bacteria and other achlorophyllous microorganisms bind it in the mineral bodies which form them.

Possible Participation of Green Algae in Agglomerations
of Hydrates of Iron Oxides

In studying aggregations of hydrates of iron oxides in the weathered crusts of granite from the nival region and in the lustrous lacquered surfaces of high-mountain varnish, we as a rule detected in them the decomposed cells of green algae stained by iron oxides, and in spots we found abundant aggregations of tiny diatom algae.

Under laboratory conditions, we attempted to explain whether green algae participate in the accumulation of hydrates of iron oxides, or whether the fact that they are confined to varnish crusts is associated merely with the better humidifying conditions of these surfaces.

Small but whole pieces of granite were taken for the experiment, placed in a Heitler medium (not containing iron), and inoculated with green algae of the genus Chlorococcum isolated from crusts of high-mountain varnish.

In one and a half to two months, the abundant development of algae had begun. The algae covered the surface of the pieces of granite with an unbroken film and settled on the walls and bottom of the flasks.

After the liquid dried up, the algae agglomerations became brown, and ocherous and reddish films like the "varnish" films observed in nature formed on the surface of the pieces of granite.

Inspection of these films under the microscope showed that they consist entirely of dead algae cells which are ocherous and red-brown in color. When the cells were treated on the slide with hydrochloric acid, we found an appreciable amount of iron (according to the reaction with ammonium thiocyanate). The quantity of iron contained in the algae cells was determined by removing the pieces of granite from the flasks and then ascertaining the weight of the mass of algae remaining in the form of films on the sides and bottom of the flasks. After cinerating the algae (in the same flasks) and dissolving the ash in hydrochloric acid, the iron in the filtrate was colorimetrically determined. The twice-repeated test for iron in the algae gave about 1% of Fe_2O_3 in terms of dry weight. In the second case 0.576-0.775% of Fe_2O_3 was obtained.

On the basis of these findings, we consider it probable that green algae (possibly along with other iron-fixing organisms) play an active role in the formation of films of high-altitude varnish. The iron source, as our experiments demonstrated, may be iron-containing minerals entering into the composition of the granite.

Conclusions

1. Microorganisms living on rocks in the nival region of the Central Tien-Shan may use various primary minerals as sources of elements necessary to life. Muscovite, biotite, orthoclase, serpentine, apatite, and a complex of minerals entering into the composition of granite may replace missing elements in nutrient media (potassium, phosphorus, magnesium, etc.) without harm to the development of the microorganisms.

2. In the process of microorganism growth, the minerals are decomposed and new minerals and organic minerals are formed. Diverse groups of microorganisms -- fungi, bacteria, diatom algae, and green and blue-green algae -- participate in the processes of decomposition and secondary synthesis.

3. Under conditions of insufficiency or complete lack of readymade organic substances, prototrophic or oligonitrophyll microorganisms develop: diatom algae, green and blue-green algae, and their concomitant coccoid bacteria. The complex of these microorganisms exerts a perceptible decomposing effect on minerals.

4. Violent growth of fungi of the genus Penicillium alongside the above-mentioned microorganisms proceeds under conditions where there is an adequate quantity of organic carbon and organic nitrogen. These fungi cause considerably more intense breakdown of minerals than does the combination of bacteria and algae.

5. The destructive effect of fungal microflora on minerals is associated with the excretion of various organic acids by the fungi. Some of these acids are dark in color and are insoluble in water, but are soluble in alkalies and are similar in their properties to the humic and fulvic acid fractions of soil humus.

In all the cases which we investigated, a fraction similar to the fulvic acids predominated in the composition of the organic acids. When the "fulvic acids" act on minerals, the latter apparently decompose.

6. Based on their degree of susceptibility to the destructive action of microorganisms, the minerals and rocks investigated may be arranged in the following descending series: muscovite, biotite, orthoclase. Granite is also subject to appreciable breakdown.

7. The formation of secondary clayey minerals, calcite, and amorphous silica proceeds along with the breakdown of the primary minerals. Amorphous silica is present chiefly as skeletons of diatom algae.

8. Green and blue-green algae and possibly the coccoid bacteria accompanying them participate in the formation of secondary calcite.

9. The accumulation of hydrates of iron oxides in the weathering products is associated with the activity of green algae of the genus Chlorococcum which are able to store up to 1.0% of Fe_2O_3 in their bodies (in terms of dry substance) under conditions where iron is present only in the form of primary minerals.

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